METHOD AND APPARATUS FOR UNLOADING GELS FROM AN ISOELECTRIC GEL TUBE ONTO A GEL SLAB

Cross-Reference to Related Application

[0001] This application is a continuation-in-part application of Serial No. 09/654,131, filed September 1, 2000 for "Method and Apparatus for Unloading Gels From Isoelectric Gel Tubes", which is hereby incorporated by reference in its entirety.

Field of the Invention

[0002] The present invention is directed to a method and apparatus for automatically unloading an isoelectric focusing gel from a tube onto a surface, and particularly onto the end of a gel slab. More particularly, the invention is directed to a method and apparatus for unloading a gel from a tube as a continuous bead and guiding the gel onto the end of a second dimension gel slab for conducting a second dimension electrophoresis separation. The invention is also directed to a method and apparatus for guiding an isoelectric focusing gel onto the edge of a gel slab.

Background of the Invention

[0003] Genomes provide the sequence information required to construct proteins that are the working parts of living cells. Genomes and genes are linear constructs composed of four different nucleotides arranged in triplet condons that specify the order and identity of the approximately 20 different amino acids that make up proteins. The nucleic acids are chemically very similar, and are arranged in very

long contiguous sequences with intervening non-coding regions. For analysis, nucleic acids must be cut up into fragments of tractable length using shearing forces or restriction enzymes which cut the nucleic acid at specific known sites.

[0004] Proteins are made of amino acid subunits that have a range of different isoelectric points, molecular weights, and solubility or hydrophobicity characteristics. The synthesized peptides have exactly defined lengths, and roll up or are assembled into proteins of well defined molecular weights. The estimated 100,000 different primary proteins in man have a range of charge densities and isoelectric points, solubilities, and surface characteristics not found in nucleic acids. Further, proteins have a range of surface conformations which mediate specific interactions between proteins, between proteins and nucleic acids, and, in the form of enzymatically active sites, between low molecular weight metabolites, and all the various types of macromolecules found in cells and foodstuffs. Proteins are the molecular machines that carry out the panoply of syntheses, disassemblies and degradations, immunochemical defense reactions, and paratactic interactions that underlie the assembly of membranes and subcellular organelles.

[0005] There is a need for analytical methods that allow a large fraction of the total number of proteins present in a cell or tissue to be detected and quantitated. The quantitative analysis of large sets of proteins that have such a wide variety of functions, sizes, conformation, activities, solubilities, and charge characteristics is both a centrally important challenge, and an exceedingly difficult problem. The problem is rendered even more difficult by the requirement that analysis detecting thousands of proteins per analyses be done in parallel on relatively large numbers of samples in

a reasonable time to do experimental toxicological and pharmacological studies.

[0006] The electrophoretic mobility of a non-denatured protein is a function of the surface charges of either the monomeric protein or the sum of the surface charges of the subunits, and these are generally used under rate-zonal conditions, i.e., under conditions where the proteins move through a gel or other support at one pH. The distance traveled is a function of the charge to mass ratio, and a function of electrophoresis time. Second dimension separations are done in gradient gels of decreasing pore size such that proteins move until movement essentially ceases as the protein reach pore sizes that prevent further movement. Experimental attempts to develop two dimensional methods based on these parameters using non-denaturing conditions have not yielded the resolution required.

[0007] Two-dimensional methods involving denaturing conditions have been explored and widely adopted. The initial separation is done in concentrated urea in the presence of ampholytes which are a heterogeneous mixture of synthetic polymers having wide variation in the ratio of acidic to basic groups. When these are subjected to an electrical field in a gel, the ampholytes sort themselves out into a continuous series based on the isoelectric point. Proteins move along the gel until they reach their own isoelectric point and stop. Further, since the proteins are denatured and unrolled, their isoelectric points reflect the sum of all of the charged groups in the protein, whether previously external or internal in the native state. The isoelectric point determination in such a separation can be calculated from the amino acid composition of the protein, and is a valuable parameter for protein classification.

[0008] The second dimensional separation is based on the length (and hence the mass) of the unrolled denatured protein and takes place in the following way. Proteins from the isoelectric separation are exposed to a highly charged detergent which has attached the longest paraffin chain which will remain extended in solution, and not fold back on itself. Sodium dodecyl sulfate (SDS) is the detergent of choice, and in solution will uniformly coat unrolled polypeptide chains, and attach to them by hydrophobic linkages, leaving the highly charged sulfate groups on the surface. The result is particles of approximately rod shape having approximately equal charge-to-mass ratios. Particles having equal charge-to-mass ratios move at the same rate in electrical fields, so that all proteins covered with SDS should have equal mobility in solution. However, if electrophoresis of such particles is done in a microporous gel, then larger particles will be retarded relative to smaller ones.

[0009] In practice, the resolutions of these two separate methods are quite high. At least 150 proteins can be resolved from a suitable mixture by isoelectric focusing, and an equal number resolved from a suitable protein mixture by SDS electrophoresis. If the two processes can be mated together in a two-dimensional array, the final resolution should be the product of the resolution of the two methods separately, i.e., 150^2 or 22,500. Experimentally, as many as 5,000 proteins have been resolved in large two-dimensional electrophoresis gels, and the theoretical resolution of current electrophoresis as calculated from spot sizes, and the number of spots which could theoretically be packed into the gel area used is around 30,000.

[0010] It is quite evident that a key step in the high-resolution two-dimensional electrophoresis technique using isoelectric focusing followed by SDS electrophoresis in the second dimension is mating the

two methods together without the loss of resolution inherent in collecting and separately analyzing fractions.

[0011] Experimentally, isoelectric focusing is done under temperature controlled conditions in glass tubes (ISO tubes) having an internal diameter of approximately 0.5-2 mm, and approximately 30 cm long. ISO tubes are then attached to a small syringe full of water or buffer solution, and the gels extruded by hand along the top of a second-dimension gel cast between two glass plates. An empty space is typically formed between the top of the gel and the top of the plates. The gels are carefully extruded into this space by a double movement in which the syringe plunger is moved to extrude the gel as the ISO tube containing the gel is moved laterally along the top of the second dimension gel. This movement requires considerable skill, and many gels are broken as they are extruded and moved into place. It is further evident that different portions of the extruded gels may be stretched differently, causing distortion in the final 2D pattern. A further difficulty is that this step is the most variable and time consuming one in present programs aimed at automating the entire 2D process, in which batches of analyses varying from 10-60 are run in parallel. The 2D protein analysis has become a core analytical method in pharmacology and toxicology, and mass spectrometric analysis and identification of proteins in spots from 2D gels has become routine and essential. Accordingly, there is a continuing need in the industry for a system and method for automatically unloading large sets of gels from isoelectric focusing gel tubes directly onto second dimension gels with minimal distortion or breakage.

Summary of the Invention

[0012] The present invention is directed to a method and apparatus for unloading a gel from a tube. More particularly, the invention is directed to a method and apparatus for unloading and guiding an isoelectric gel from a gel tube onto a gel slab or other work surface.

[0013] Accordingly, a primary object of the invention is to provide a method and apparatus for unloading a substance, and particularly a gel, from a tube substantially without distortion of the substance.

[0014] A further object of the invention is to provide a method and apparatus for unloading an isoelectric gel from a gel tube in a substantially uniform and controlled manner.

[0015] Still another object of the invention is to provide a method and apparatus for guiding a gel onto the edge of a gel slab between two support plates of a second dimension gel cassette.

[0016] Another object of the invention is to provide an apparatus for removing a gel body from a cylindrical tube substantially without compressing or elongating the gel body.

[0017] A further object of the invention is to provide an apparatus for removing a substance from a cylindrical tube onto a surface by passing a plunger rod through the tube at a substantially uniform speed.

[0018] A further object of the invention is to provide a method and apparatus for unloading a gel from a cylindrical tube using a plunger rod mounted in a fixed position where one end of the tube slides onto the plunger rod to discharge the gel from the opposite end of the tube.

[0019] Another object of the invention is to provide a method and apparatus for unloading a substance from a tube at a controlled rate where the tube is moved along the surface at a controlled rate to discharge of the gel from the tube uniformly onto a surface.

[0020] A further object of the invention is to provide a method and apparatus for discharging a gel from a tube using a flexible plunger member having a width greater than an internal diameter of the tube where the plunger member is fitted into one end of the tube and pushed along the length of the tube.

[0021] Still another object of the invention is to provide an apparatus for unloading a gel from a tube onto a surface where the apparatus moves the tube across the surface at a substantially constant speed and constant angle with respect to the direction of movement of the tube.

[0022] A further object of the invention is to provide an apparatus for unloading a gel from a plurality of tubes where the apparatus includes a movable support for the tubes, a plunger rod associated with each tube, and a stationary support coupled to one end of the rod, where the movable support moves toward the stationary support to slide one end of the tubes over the respective plunger rod and to unload the gel from the opposite end of the tubes.

[0023] Another object of the invention is to provide a guide member having at least one inclined guide surface for guiding a first dimension gel into the end of a second dimension electrophoresis gel.

[0024] A further object of the invention is to provide a guide assembly having a body with a first inclined guide surface and a second guide surface for guiding a first dimension electrophoresis gel into a respective second dimension gel cassette.

[0025] Still another object of the invention is to provide a guide assembly for guiding sample gels into a respective second dimension gel cassette, where the assembly includes a plurality of parallel guide members, each of the guide members having opposite guiding

surfaces, which cooperate with guiding surfaces of an adjacent guide member for guiding the sample gels.

[0026] A further object of the invention is to provide a guide assembly for guiding sample gels into a respective second dimension gel cassette where the assembly includes a plurality of parallel guide members coupled to a support plate.

[0027] The objects and advantages of the invention are further attained by providing an apparatus for guiding a cylindrical gel between two plates of a second dimension electrophoresis gel cassette. The device includes a pair of inclined surfaces positioned at the end of the gel cassette and converging toward the space between the plates. The bottom end of the inclined surfaces are preferably spaced apart a distance substantially equal to the spacing between the plates of the cassette. The top end of the inclined surfaces are spaced apart a distance at least equal to the width of the gel from the gel tube.

[0028] In one embodiment of the invention, an assembly is provided for guiding a plurality of tubular shaped gels into a respective gel slab cassette. A plurality of the cassettes are supported in a tray and oriented vertically with the gel receiving end oriented along the horizontal top end. The assembly is positioned onto the top end of the cassettes for guiding the tubular gels into the open end of the cassettes. The assembly includes a plurality of guide members having a substantially triangular shape and having a dimension to rest on the top end of two adjacent glass plates of two adjacent cassettes. The guide members have opposite inclined side faces that converge at an apex at a top end and terminate at a bottom end at the top edge of the glass plate. Each of the guide members are coupled together at their longitudinal ends to fix the guide members in a uniformly spaced-apart array.

[0029] The objects and advantages of the invention are basically attained by providing an apparatus for unloading a substance from a tube. The apparatus includes an electrophoresis gel slab having a longitudinal edge and a guide member coupled to the longitudinal edge. The guide member has at least one inclined guiding surface for guiding the gel to the longitudinal edge of the gel slab. A first support assembly is provided for supporting at least one tube. The at least one tube has an axial passage, a first open end and a second open end. The first end of the tube is coupled to the first support. A second support is spaced from the first support. A plunger is provided with a first end coupled to the second support and a second end axially aligned with the axial passage of the at least one tube. A drive assembly slides the at least one tube onto the plunger to unload the gel from the second end of at least one tube onto the guiding surface of the guide member.

[0030] The objects and advantages of the invention are yet further attained by providing a guide assembly for guiding a gel having a longitudinal dimension into a space between first and second support plates of a second dimension electrophoresis gel cassette. The guide assembly comprises a first guide member having a bottom end dimensioned to overlie a top end of the first support plate and a top end spaced from the bottom end. The first guide member includes an inclined surface which extends between the top end and the bottom end. A second guide member has a top end and a bottom end dimensioned to overlie a top end of the second support plate. The second guide member has a guiding surface which extends between the top end and the bottom end. The first inclined surface of the first guide member and the guiding surface of the second guide member

converge toward the space between the first and second support plates.

[0031] The objects and advantages of the invention are further attained by providing an assembly for guiding a plurality of gels onto an edge of a respective second dimension electrophoresis gel. The assembly comprises a plurality of guide members arranged in a substantially parallel spaced-apart relation. Each of the guide members has a body with a top end, a bottom end and a guiding surface extending between the bottom end and the top end. The bottom ends of the guide members are spaced apart a distance substantially equal to a space between adjacent second dimension electrophoresis gels.

[0032] The objects and advantages of the invention are yet further attained by providing an assembly for loading a sample onto the end of a second dimension electrophoresis gel. The assembly comprises a plurality of second dimension electrophoresis gel cassettes supported in a spaced apart upright orientation. Each of the cassettes has a top end for receiving the sample. A guide assembly is positioned on the top end of the gel cassettes. The guide assembly has a longitudinal length with guiding surfaces extending toward the top end of the cassettes for guiding the sample onto the top end of the cassette. A supply device is provided for supplying the sample along the longitudinal length of the guide assembly.

[0033] The objects, advantages and salient features of the invention will become apparent to one skilled in the art in view of the following detailed description of the invention in conjunction with the annexed drawings which form a part of this original disclosure.

Brief Description of the Drawings

[0034] The following is a brief description of the drawings, in which:

[0035] Figure 1 is a perspective view of the unloading apparatus of the invention;

[0036] Figure 1A is a side elevational view of the apparatus of Figure 1;

[0037] Figure 2 is a top view of the apparatus of Figure 1;

[0038] Figure 3 is a cross-sectional side view of the apparatus taken along line 3-3 of Figure 2;

[0039] Figure 4 is an end view of the rack in one embodiment of the invention;

[0040] Figure 5 is a front view of the gel tube rack in one embodiment of Figure 3;

[0041] Figure 6 is a side view of the gel tube rack of Figure 5;

[0042] Figure 7 is a top view of the gel tube rack of Figure 5;

[0043] Figure 7A is a top view of a gel tube rack in another embodiment;

[0044] Figure 7B is a side view of the rack of Figure 7A;

[0045] Figure 8 is a side view of the assembly showing the gel slabs and gel tube rack coupled to the unloading apparatus;

[0046] Figure 9 is an end view of the gel slabs monitored in the supporting tray;

[0047] Figure 10 is a schematic side view showing the gel being unloaded onto the edge of a gel slab;

[0048] Figure 11 is an end view showing the bead of unload gel on the edge of the gel slab;

[0049] Figure 12 is side view of the assembly of Figure 8 showing the position of the gel tubes after the gel is unloaded;

[0050] Figure 13 is a top view of the assembly of Figure 8;

[0051] Figure 14 is a top view of the assembly of Figure 8 showing the position of the gel tubes after the gel is unloaded;

[0052] Figure 15 is a side view in cross-section of an unloading device in a second embodiment of the invention;

[0053] Figure 16 is an exploded perspective view of the support tray, second dimension electrophoresis gel cassettes and guide members;

[0054] Figure 17 is a perspective view showing the gel cassettes supported in the support tray;

[0055] Figure 18 is an enlarged perspective view of a guide member;

[0056] Figure 19 is a perspective view showing the guide members on the gel cassettes and showing a first dimension electrophoresis gel being unloaded from a gel tube and guided onto a gel cassette;

[0057] Figure 20 is an end view in cross-section showing the first dimension gel being guided onto the open end of the gel cassette;

[0058] Figure 21 is a side view showing the guide assembly positioned on the gel cassettes and a first dimension gel being unloaded and guided onto the open end of the gel cassette;

[0059] Figure 22 is a perspective view of a guide assembly showing a plurality of guide members coupled to the support plate;

[0060] Figure 23 is a rear view of the guide assembly of Figure 22;

[0061] Figure 24 is a front view of the guide assembly of Figure 22;

[0062] Figure 25 is a top view of the guide assembly of Figure 22;

[0063] Figure 26 is a side view in partial cross-section showing the automated gel unloading device for unloading gels onto the guide assembly;

[0064] Figure 27 is a perspective view of the guide assembly in another embodiment; and

[0065] Figure 28 is an end view in partial cross-section showing a gel being guided onto the edge of a gel slab.

Detailed Description of the Invention

[0066] The present invention is directed to a method and apparatus for unloading and dispensing a substance from a tube. In particular, the invention is directed to a method and apparatus for unloading a substance from a tube in a controlled and uniform manner. The invention is also directed to a guide assembly for guiding a gel from an electrophoresis gel tube onto the edge of a second dimension gel slab.

[0067] The method and apparatus of the invention are primarily directed to transferring an isoelectric focusing gel from a gel tube onto the edge of a gel slab in a two dimensional separation process. A protein sample is subjected to a first dimensional electrophoresis separation as known in the art. The electrophoresis first dimension separation utilizes a cylindrical tube that is typically made of glass and has an internal diameter of about 0.5 mm to about 2 mm and a length of about 30 cm. The tube is filled with an isoelectric focusing gel, such as an acrylamide gel. The protein sample is applied to one end of the tube while each end of the tube is in contact with a buffer solution to define a pH gradient along the length of the tube. An electric potential is applied to each end of the tube whereby the proteins migrate through gel. The gel must then be removed from the tube and placed on the end of the gel slab to conduct the second dimension electrophoresis separation. It is essential for consistent results that the gel be transferred intact with minimal distortion of the gel body.

[0068] The apparatus of the invention is constructed to unload a gel from a tube onto a surface, and particularly a gel slab, without breaking the gel. The apparatus unloads the gel from the tube at a controlled rate while moving the end of the tube across the surface at

a rate complementing the discharge rate so that the gel is unloaded in a controlled manner. It is particularly desirable to unload an isoelectric gel body from the gel tubes in a uniform manner to avoid elongation or compression in portions of the gel body. In embodiments of the invention, the apparatus is able to unload the gel with uniform elongation or compression as desired throughout the entire length of the gel.

[0069] The apparatus of the invention is particularly constructed to remove an isoelectric focusing gel from the tube directly onto the edge of the gel slab for further separation of the proteins. Since the different proteins are spaced along the length of the gel, it is necessary to remove the gel without breaking the gel. The apparatus unloads the gel from the tube without distortion or twisting. Although the invention is primarily concerned with unloading gels, it will be understood that the apparatus and method are suitable for unloading a number of substances. For example, the apparatus and method can be used to unload capillary electrophoresis gels, ringing gels, DNA containing gels, paste-like, rubber-like or viscous creams. The apparatus can also be used to clean and remove gel residues from tubes after the bulk of the gel is transferred to a gel slab.

[0070] Referring to the drawings, the unloading apparatus 10 of the invention includes a first support assembly 12, a second support assembly 14, and a plurality of plunger rods 16.

[0071] First support assembly 12 is mounted on a base 18 as shown in Figures 1 and 1A. Base 18 includes a bottom rail 20, a pair of front posts 22 and two rear posts 24. A guide rail 26 extends from each front post 22 to the respective rear post 24. Preferably, two parallel guide rails 26 are provided on each side of base 18.

[0072] First support assembly 12 includes a mounting plate 28 and a carriage 30. Carriage 30 has a longitudinal dimension corresponding to the width of base 18. A first end 32 of carriage 30 is connected to a bracket 34. Bracket 34 has two downwardly extending legs 36 with axially aligned bores 38. Bores 38 are dimensioned to complement guide rail 26 so that bracket 34 is able to slide on rail 26. Preferably, guide rail 26 is a substantially cylindrical shaped rod, although rail 26 can be any suitable shape. A second end 40 of carriage 30 includes a second bracket 42. Bracket 42 includes a pair of legs 44 with axially aligned bores 46 in a manner similar to the first bracket 34. Bracket 42 as shown in Figures 1 and 2 has a length greater than the first bracket 34. Second bracket 42 includes an end 48 forming a stop member as discussed hereinafter in greater detail. Brackets 34 and 42 are slidably mounted on a respective guide rail 26 as shown in Figure 2 for sliding movement along the length of guide rails 26.

[0073] Bracket 34 and bracket 42 each include an end wall 50 and 52, respectively, extending in a generally upright direction. End walls 50 and 52 are united in a plane substantially parallel to the longitudinal dimension of guide rails 26. Mounting plate 28 is a substantially rectangular shaped plate having opposite ends coupled to a respective end wall 50 and 52 by screws 53 as shown in Figure 1. Mounting plate 28 has a front face 54 and a rear face 56. A plurality of spaced apart openings 58 extend between front face 54 and rear face 56 as shown in Figure 2. Openings 58 are uniformly spaced apart and aligned in a row extending between bracket 34 and bracket 42.

[0074] As shown in Figures 1 and 2, each end of mounting plate 28 includes a bore 60 extending between front face 54 and rear face 56.

A rigid guide rod 62 is fitted into bores 60 to extend outwardly from rear face 56. In preferred embodiments, each guide rod 62 is fixed to mounting plate 28 and is substantially immovable with respect to mounting plate 28 and carriage 30. Each guide rod 62 extends from rear face 54 along an axis substantially parallel to the axis of openings 58 in mounting plate 28. Guide rod 62 can be press fitted into bores 60 or secured in place by a screw or other fastener.

[0075] Second support assembly 14 includes a bracket 68 coupled to each guide rail 26 and a support bar 70. Each bracket 68 includes a leg 72 having a bore 74 complementing the dimension of guide rail 26. Guide rail 26 extends through each bore 74 for supporting brackets 68. The position of each bracket 68 can be adjusted along the length of guide rail 26. In preferred embodiments, brackets 68 include a coupling member such as a set screw 76 for fixing the position of bracket 68 on the respective guide rail 26.

[0076] Brackets 68 each include an upstanding end wall 78 extending upwardly from leg 72. An elongated slot 80 is provided in each end wall 78 as shown in Figures 1 and 1A. Slot 80 has a longitudinal dimension extending in a generally vertical direction. In the embodiment illustrated slot 80 is oriented at an angle with respect to guide rail 26 to extend at an incline toward first support 12. In further embodiments as discussed thereinafter in greater detail slot 80 can be oriented perpendicular to guide rail 26 or slightly inclined away from first support 12.

[0077] Support bar 70 extends between brackets 68 and includes a front face 82 and a rear face 84. A plurality of spaced apart openings 86 extend between front face 82 and rear face 84. Each end of support bar 70 includes a pinion 88 and a bearing 90. Bearing 90 is preferably a roller bearing that is dimensioned to fit and slide within

slot 80 as shown in Figure 2. Each end of support bar 70 also includes a bore 92 extending between front face 82 and rear face 84. In preferred embodiments, a bushing 94 having an axial passage 96 is fitted within each bore 92. Axial passage 96 of each bushing 94 is dimensioned to receive a respective guide rod 62. Guide rods 62 are dimensioned to slide through axial passage 96 of each bushing 94.

[0078] As shown in Figure 2, a plurality of plunger rods 16 have a first end 98 received in a respective opening 86 of support bar 70. In the embodiment illustrated, support bar 70 has a top face 100 having a threaded bore 102 extending into the axial passage of each opening 86. A set screw 104 is threaded into the bores to couple the first end 98 of each plunger rod 16 to support bar 70. Plunger rods 16 include a second end 106 received in a corresponding opening 58 of mounting plate 28. The location of a second end 106 of plunger rods 16 can be individually adjusted in opening 58 of mounting plate 28 by loosening screws 104 and adjusting the position of each plunger rod 16 in support of bar 70.

[0079] In the illustrated embodiment, brackets 68 are fixed to guide rails 26 during the use of the apparatus. The linear movement of support bar 70 and plunger rod 16 in the direction of guide rails 26 is limited by the incline of slot 80 with respect to guide rail 26. Carriage 30 and mounting plate 28 are slidable along guide rails 26 from an extended position shown in Figure 1 to a retracted position toward second support assembly 14. The angle of mounting plate 28 and guide rods 62 remain constant with respect to guide rail 26 as carriage 30 slides along guide rail 26.

[0080] A drive assembly 108 is provided for sliding carriage 30 and mounting plate 28 along guide rails 26 at a constant speed. The illustrated embodiment of drive assembly 108 includes a motor 110

operatively connected to a first end 112 of a threaded rod 114. Threaded rod 114 extends substantially parallel to guide rails 26. In the embodiment illustrated, motor 110 is mounted on a cross support 116 extending between rear posts 24. A coupling 118 is connected to carriage 30 as shown in Figure 1. Coupling 118 includes an internally threaded bore for coupling with a second end 120 of threaded rod 114. Motor 110 is energized to rotate threaded rod 114 about its axis and move coupling 118 and carriage 30 along the axis of threaded rod 114. In a preferred embodiment, motor 110 is a reversible motor to rotate threaded rod 114 in different directions to selectively move carriage 30 toward or away from second support 14 depending on the direction of rotation of threaded rod 114.

[0081] In the illustrated embodiment, motor 110 is mounted adjacent second support assembly 14 at the rear of assembly 10. In further embodiments, motor 100 can be mounted toward the front end of base 18 with threaded rod 114 extending toward the rear end of base 18. Alternative drive assemblies can also be used, such as, for example, a chain or gear drive.

[0082] Apparatus 10 is used in conjunction with isoelectric focusing gel tubes 122. Gel tubes 122, as shown in Figure 5, have a substantially cylindrical shape with an axial passage 124, a first open end 126 and a second open end 128. Gel tubes 122 are mounted in a tube support member, such as a rack 130 as shown in Figure 5. In the illustrated embodiments, gel tubes 122 have a cylindrical shape. In further embodiments, gel tubes 122 can have a non-circular cross-section such as an oval, square or rectangular shape. As used herein, the term "tube" is intended to refer to an elongated hollow body and is not limited to cylindrical shaped tubes.

[0083] Rack 130 is a support suitable for use in an electrophoresis tank during a first dimension electrophoresis separation process. In one embodiment of the invention, rack 130 includes side walls 132 and a lower brace 134 extending between side walls 132. A plurality of spaced apart openings 136 dimensioned to receive gel tubes 122 are provided in lower brace 134. A trough assembly 138 is coupled to the top end of side walls 132 by screws 140 or other suitable fasteners. Trough 138 includes side walls 142 and a bottom wall 144. Bottom wall 144 includes a plurality of spaced apart openings 146 axially aligned with openings 136 and lower brace 134. An upper brace 148 extends between side walls 132 directly below bottom wall 144 of trough 138. Upper brace 148 also includes a plurality of spaced apart openings 150 aligned with openings 146 and 136. Side walls 142 and bottom wall 144 of trough 138 define a chamber 152 for containing a buffer solution suitable for use in conducting a first dimension electrophoresis separation.

[0084] In the illustrated embodiment, rack 130 supports two rows of gel tubes 122. The rack 130 shown in Figures 5-7 is an example of a suitable rack for supporting gel tubes 122, although it will be understood that other structures can be used. In one embodiment, a gel tube rack 130' as shown in Figures 7A and 7B includes a single row of gel tubes 122'. Rack 130' is similar to rack 130 and is coupled to unloading apparatus 10 in a similar manner with plunger rods 16 aligned with gel tubes 122'.

[0085] Referring to Figure 5, trough 138 includes a top face 154. Top face 154 includes two internally threaded bores 156 adjacent each side edge 158. As shown in Figure 3, mounting plate 28 includes complementing holes 160 aligned with threaded bores 156 of top face 154. Screws 162 extend through holes 160 in mounting plate 28 and

are threaded into bores 156 of top face 154 to couple rack 130 to mounting plate 28. Threaded bores 156 in top face 154 are oriented with respect to holes 160 in mounting plate 28 to align openings 58 of mounting plate 28 and respective plunger rod 16 with a row of gel tubes 122. It will be appreciated that openings 58 in mounting plate 28 and plunger rod 16 are spaced apart a distance corresponding to the spacing of gel tubes 122 in rack 130. Preferably, the number of gel tubes 122 in rack 130 correspond to the number of plunger rods 16 in apparatus 10. In the embodiment shown in the drawings, a single row of plunger rods 16 are provided and aligned with one row of gel tubes 122 in rack 130. Preferably threaded bores 156 in top face 154 of rack 130 are positioned so that rack 130 can be inverted with respect to mounting plate 28 to align plunger rods 16 with a selected row of gel tubes 122. In this manner, each row of gel tubes 122 can be unloaded by inverting rack 130 and reinstalling on mounting plate 28.

[0086] As discussed hereinafter in greater detail, apparatus 10 is primarily intended for use in transferring the isoelectric focusing gel from a respective gel tube 122 onto a gel slab 164 for conducting a second dimension electrophoresis separation as known in the art. Gel slabs 164 in preferred embodiments of the invention include a layer of an electrophoresis focusing gel 166 sandwiched between two glass plates 168 as shown in Figure 9. Typically, a spacer 170 in the form of a narrow glass strip is positioned adjacent each end of glass plates 168 as shown in Figure 8 to provide uniform spacing of glass plates 168. Gel slabs 164 are supported in a tray 172 that is coupled to apparatus 10 adjacent front post 22. Tray 172 includes a bottom wall 174 and spaced apart ribs 176 extending substantially perpendicular from bottom wall 174 a sufficient distance to support gel slabs 164.

Preferably, ribs 176 are spaced apart a distance corresponding to the thickness of gel slabs 164 to support gel slabs 164 in an upright fashion as shown in Figure 9. Ribs 176 are dimensioned to position gel slabs 164 in a spaced apart relation corresponding to the spacing between gel tubes 122 of rack 130 as shown in Figures 13 and 14. Gel slabs 164 are oriented in an upright position parallel to gel tubes 122 and with a substantially horizontal upper edge 178.

[0087] Gel tubes 122 containing an isoelectric focusing gel 180 are fitted into rack 130 for use in a first dimension electrophoresis separation of a biological sample. After the electrophoresis separation is completed, rack 130 with gel tubes 122 still attached is coupled to mounting plate 28 by screws 162. As shown in Figure 8, mounting plate 28 is substantially perpendicular to the longitudinal axis of gel tubes 122 in rack 130, and plunger rods 16 are coaxially aligned with a respective gel tube 122. Gel slabs 164 are positioned in tray 172 and aligned with a corresponding gel tube 122. As shown in Figures 8 and 13, first end 126 of gel tubes 122 are aligned with openings 58 in mounting plate 28 and a respective plunger rod 16. Second end 128 of gel tubes 122 are positioned on or slightly above upper edge 178 of gel slab 164 adjacent a first end 182. As shown in Figure 8, mounting plate 28 is oriented at an angle with respect to guide rails 26 and upper surface 178 of gel slabs 164. In embodiments of the invention, mounting plate 28 can include a coupling assembly such as an elongated slot and screw member for adjusting the angular position of mounting plate 28 with respect to upper edge 178 of gel slabs 164. Carriage 30 is moved to the extended position shown in Figure 8 by actuating motor 110 to position second end 128 of gel tube 122 at first end 182 of gel slab 164. Second support assembly 14 is then

adjusted on guide rails 26 until second end 106 of plunger rods 16 are positioned at first end 126 of gel tubes 122.

[0088] As shown in Figure 10, plunger rods 16 have an outer dimension corresponding substantially to the dimension of axial passage 124 of gel tubes 122. In practice, plunger rods 16 have a diameter slightly less than the internal diameter of axial passage 124 to be able to slide through axial passage 124 without interference. It has been found that plunger rods 16 are able to express gel 180 from gel tubes 122 onto gel slabs 164. However, the variations in texture of gel 180 in gel tubes 122 can result in pieces of the gel adhering to the inner surface of tube 122 being broken away and separated from the gel body as the gel is unloaded from the gel tube. In one preferred embodiment of the invention, a plunger member 184 is placed in the second end 126 of gel tubes 122 between gel 180 and second end 106 of plunger rods 16 as shown in Figure 10. Plunger member 184 is preferably made of a resilient material having a diameter slightly greater than the internal diameter of axial passage 124 of gel tube 122 to prevent pieces of the gel from adhering to the surface of the tube. In preferred embodiments, plunger member 184 is a ball shaped member made of a silicone rubber. The silicone rubber ball has an outer dimension that is able to contact the inner surface of axial passage 124 and is able to pass through axial passage by the force applied by plunger rod 16. Plunger member 184 in combination with plunger rods 16 are able to consistently unload the gel as a continuous body with little or no tearing, breaking or distortion of the gel.

[0089] Rack 130 is coupled to mounting plate 28 and gel tubes 122 are aligned with a respective gel slab 164. Motor 110 is then actuated to rotate threaded rod 112. Rotation of threaded rod 112 pulls

carriage 30 and mounting plate 28 at a constant speed toward second support assembly 14. First end 98 of plunger rods 16 are coupled to support bar 70 so that gel tubes 122 slide onto plunger rods 16 and express and unload gel 80 from gel tubes 122 onto upper edge 178 of gel slabs 164. Motor 110 is operated at a speed to unload gel 80 from gel tubes 122 at a controlled and uniform rate. As shown in Figure 12, guide rods 62 slide through bore 92 of support bar 70 to maintain plunger rods 16 in axial alignment with gel tubes 122 and to maintain gel tubes 122 at a constant angle with respect to gel slabs throughout the unloading process. Motor 110 is operated until carriage 30 travels a distance sufficient to unload gel 180 onto gel slabs 164 as shown in Figures 10 and 11. At that time, gel slabs 164 are removed from tray 172 and transferred to a suitable second dimension electrophoresis separation apparatus.

[0090] As shown in Figures 8 and 12, the angle of mounting plate and guide rods 62 with respect to guide rails 26 causes support bar 70 and bearings 90 to slide within slot 80 of end walls 78 of brackets 68. In the embodiment illustrated, slot 80 is oriented at an incline so that support bar 70 moves away from gel slabs 164 as carriage 30 is moved toward second support assembly 14. It will be understood that the actual angle of slot 80 will determine the amount of movement of support bar 70 during movement of carriage 30. In this embodiment, plunger rods 16 are moved away from gel slabs 164, as carriage and gel tubes 122 are moved toward second support 14 so that the end of gel tubes 122 slide along the top edge of gel slabs 164 at the same or a slightly faster rate than the rate that the gel body 180 is being unloaded. In preferred embodiments, the ratio of the rate of unloading the gel to the rate of the movement of the gel tubes across the gel slabs is about 1 to 1. This coordinated movement of plunger

rods 16 and gel tubes 122 result in gel 80 being slightly stretched or elongated as it is unloaded from gel tube 122. In further embodiments, slot 80 can be oriented substantially perpendicular to guide rails 26 so that gel 80 in gel tube 122 is unloaded onto gel slabs 164 with substantially no elongation or compaction during unloading. In still further embodiments, slot 80 can be oriented to move support bar 70 toward gel slabs 164 during movement of carriage 30 to compress gel 180 as it is unloaded from the gel tubes 122.

[0091] A second embodiment of the invention as shown in Figure 15 is a manually operated unloading device 200. Unloading device 200 is a hand held device having a housing 202 with a generally cylindrical shape with a first end 204 and a second end 206. First end 204 has a flange 208 extending radially outward a distance sufficient for an operator to grip device 200.

[0092] Housing 202 has an axial passage 210 extending between first end 204 and second end 206. Axial passage 210 has a first cylindrical section 212 extending from second end 206 and is dimensioned to receive a gel tube 214. A second cylindrical section 216 extends from first end 204 and joins first section 212 to define a stepped portion 218. A plunger rod 220 has a first end 222 extending through axial passage 210 from first end 204. A second end 224 of plunger rod 220 includes an actuator member 226.

[0093] In preferred embodiments, plunger rod 220 is a cylindrical shaped member made from metal or other sufficiently rigid material to expel and unload the gel from gel tube 214. Typically, plunger rod 220 has an outer dimension to slide easily through gel tube 214 and apply a uniform pressure on plunger member 220.

[0094] Gel tube 214 has a first open end 217, second open end 219, and an axial bore 221 containing a gel. As shown in Figure 15, gel

tube 214 has open end 217 fitted into first section 212 so that the end seats against stepped portion 218 and holds gel tube 214 in housing 202. In preferred embodiments, gel tube 214 is coupled to housing 202 by a friction fit. A resilient plunger member in the form of a spherical rubber ball 228 is placed in the end of gel tube 214 as in the previous embodiments. Plunger rod 220 is actuated by manually pressing actuator member 226 while the operator holds housing 202. The open end 219 of gel tube 214 is moved across the end of a gel slab 230 while discharging an IEF gel material 232 onto gel slab 230. As in the previous embodiment, ball 228 in combination with plunger rod 220 effectively discharges gel 232 without distortion. Ball 228 applies a substantially uniform pressure across the diameter of gel 232 in gel tube 214 to unload the gel as a continuous line.

[0095] In the embodiment shown, gel tube 214 is dimensioned to fit securely in housing 202. In alternative embodiments, a rubber-like grommet or gasket can be provided in axial passage 210 to secure gel tube 214 in place.

[0096] Unloading device 200 is used in a method for unloading gels from a gel tube in a singular fashion onto a gel slab. In further embodiments, housing 202 can include a plurality of parallel axial passages for supporting a plurality of gel tubes. In the method of the invention, gel tube 214 is inserted into axial passage 210 and a plunger member 228 is placed in axial passage 210 and aligned with the axial bore 221 of gel tube 214. Plunger rod 220 is then inserted into axial passage 210 of housing 202 and aligned with plunger member 228 and axial bore 221 of gel tube 214. Plunger rod 220 is then actuated to push plunger member 228 through gel tube 214 to unload the gel.

[0097] In the illustrated embodiments of Figures 5-12, rack 130 includes two parallel rows of gel tubes 122. In further embodiments, gel tubes 122 can be oriented in various other arrangements. For example, a gel tube rack can be formed with recesses for supporting a plurality of gel tubes in a non-linear pattern, such as circular, square or rectangular pattern.

[0098] In another embodiment of the invention, a gel tube rack is provided with two parallel rows with recesses for supporting gel tubes where the rows are staggered with respect to each other. In this embodiment, the two rows of gel tubes are staggered so that both rows of gel tubes can be aligned with a gel slab and unloaded simultaneously. Preferably, the gel slabs are supported in a tray where the upper edges of every other gel slab is staggered to complement the staggering of the gel tubes in the rack. The unloading device includes a complementing number of plunger rods aligned with each gel tube. In this manner, two rows of gel tubes are unloaded simultaneously onto staggered gel slabs. Staggering the gel slabs provides an arrangement to separate the unloaded gels and reduce the possibility of the adjacent gels contacting each other.

[0099] The isoelectric focusing gel is expressed from the gel tube onto the end of the gel slab. Typically, it is necessary to push the cylindrical shaped gel downwardly between the plates that support the gel slab. A small tool is often used to push the gel past the top edge of the plates. The gel is very tacky so that care must be taken to avoid damaging or tearing the gel.

[0100] Referring to Figures 16-21, a guide member 250 is used in conjunction with the gel slabs and the unloading device for guiding the gel from the gel tube onto the end of the gel slab in a manner to prevent damage to the gels. Referring to Figure 16, a support tray 250

includes a plurality of spaced apart dividers 254 forming recesses 256 in tray 250. Recesses 256 have a dimension to receive and support a second dimension gel cassette 258. Preferably, recesses 256 have a width substantially equal to the width of cassette 258 to support cassettes 258 in a substantially upright orientation. In preferred embodiments, cassettes 258 are supported substantially parallel to each other and spaced apart a distance corresponding to the spacing between the gel tubes of the unloading device.

[0101] Cassettes 258 include two spaced apart supporting plates 260 supporting an electrophoresis gel 262 therebetween. Gel 262 has an upper surface 264 forming an edge spaced from a top end 266 of support plates 260 to define a recess 268 in cassette 258. Preferably, recess 268 has a dimension sufficient to receive the tubular shaped gel from the first dimension electrophoresis gel tube.

[0102] Guide members 250 have a body 270 with a longitudinal length substantially equal to the length of top end 266 of support plates 260. Body 270 in the illustrated embodiment has a first inclined guiding surface 272 and a second inclined guiding surface 274 extending between a top end 276 and a bottom end 278. As shown in Figure 16, body 270 has a substantially triangular shape defined by guiding surfaces 272 and 274 and bottom end 278.

[0103] A leg 280 extends from bottom end 278 of body 270. In the embodiment illustrated, a single leg 280 is provided and extends the longitudinal length of body 270. In alternative embodiments, leg 280 can have a length less than the length of body 270. In other embodiments, two or more legs can be provided along bottom end 270 and spaced apart a distance sufficient to support guide member 250 on a respective cassette 258.

[0104] Leg 280 is preferably spaced from a bottom edge 282 of first guiding surface 272 to form a supporting ledge 284. Ledge 284 is formed by a first side 286 of leg 280. A second side 288 of leg 280 is spaced from a bottom edge 290 of second guiding surface 274 to form a second ledge 292. Referring to Figures 19 and 21, first ledge 284 and second ledge 292 have a width corresponding substantially to the thickness of support plates 260. As shown in Figure 21, bottom edge 282 of first guiding surface 272 and bottom edge 290 of second guiding surface 274 are positioned along an inner edge 294 of support plates 260. In alternative embodiments, ledges 284 and 292 can be slightly less than the thickness of support plates 260. In other embodiments, one or both of ledges 284 and 292 can have a width greater than the thickness of support plates 260 to overhang recess 268 of cassettes 258.

[0105] As shown in Figure 19, leg 280 of guide members 250 have a width corresponding substantially to the space between adjacent cassettes 258. Guide members 250 are positioned along top end 266 of cassettes 258 with leg 280 of each guide member positioned between adjacent cassettes 258. Adjacent guide members 250 are positioned on cassettes 258 to form a substantially V-shaped trough 296 above each recess 268 of cassettes 258.

[0106] Guide members 250 are preferably made of a suitable plastic material providing a non-stick surface for the electrophoresis gel to prevent or inhibit the gel from sticking as it is unloaded from the gel tube. In one embodiment, guide members 250 are made from a plastic material having a low coefficient of friction, such as polytetrafluoroethylene. In other embodiments, guide members 250 can be made of other materials with a coating or surface layer on the guiding surfaces having a lubricity sufficient to inhibit or prevent the

electrophoresis gel from sticking to guide members 250. The non-stick guide surfaces enable the gel to slide easily and fall between support plates 260 with little or no effort and generally without the use of other tools that can damage the gel.

[0107] Guide members 250 are assembled onto cassettes 258 as shown in Figure 19. An electrophoresis gel 298 is unloaded from an electrophoresis gel tube 300 to deposit gel 298 in the V-shaped trough 296. Guiding surfaces 272 and 274 forming V-shaped trough 296 enable gel 298 to fall downwardly by gravity into recess 268 onto upper edge 264 of gel slab 262. Guide members 250 also assist in aligning gel tube 300 directly above recess 268 and prevent gel 298 from catching on the top edge 266 of support plates 260. Typically, the non-stick surfaces of first guiding surface 272 and second guiding surface 274 enable gel 298 to slide easily into recess 268 without manual assistance or manipulation of gel 298.

[0108] In another embodiment shown in Figures 22-25, a guide assembly 301 includes several guide members 250 connected to a support plate 302. Support plate 302 has a substantially planar configuration with a height less than or equal to the height of guide members 250. Support plate 302 has a longitudinal length corresponding to the length of tray 252 for cooperating with the cassettes 258 positioned in tray 252. Guide members 250 have a longitudinal end 304 that are coupled to support plate 302 by a suitable fastener 306. In one embodiment, fastener 306 is a screw 308 extending through an aperture 310 in support plate 302 and threaded into a longitudinal bore 312 in guide member 250. Preferably, support members 250 are spaced apart along support plate 302 a distance corresponding to the spacing between cassettes 258 when mounted in tray 252. In this manner, assembly 301 can be

easily positioned on a plurality of cassettes 258 while ensuring proper alignment with the cassettes 258. In the embodiment illustrated, a single support plate is provided to support guide members 250. In alternative embodiments, a support plate can be provided at each end of the guide members 250.

[0109] Individual guide members 250 can be positioned along the top edges of cassettes 258 as shown in Figure 21. Gel 298 can be unloaded from gel tube 300 using a manually operated plunger rod 314 as shown in Figures 19-21. Gel tube 300 is moved along the length of the V-shaped trough 296 between adjacent guide members 250 while unloading gel 298 onto or between the guiding surfaces 272 and 274 of adjacent guide members 250. As shown in Figures 20 and 21, guide members 250 direct gel 298 toward recess 268 and enable gel 298 to fall downwardly between support plates 260 onto top surface 264 of gel 262.

[0110] The guide assembly 301 formed by guide members 250 attached to support plate 302 is positioned on top of cassettes 258 as shown in Figure 26. Preferably, the assembly is positioned on the cassettes 258 with support plate 302 positioned at the leading edge of cassettes 258. In the embodiment illustrated in Figure 26, the automated unloading apparatus 10 as previously discussed is used to unload the gel 298 from gel tubes 300. Gel tubes 300 are initially positioned at the leading edge of cassettes 258 and the gel is unloaded as gel tube 300 is moved from the leading edge toward the trailing edge of cassette 258.

[0111] The guide assembly of the invention is particularly suitable for use with an automated gel unloading apparatus that is able to unload the gel from a plurality of gel tubes either simultaneously or sequentially. In the previous embodiments, the guide assembly is

constructed to cooperate simultaneously with several second dimension electrophoresis gel cassettes containing a gel slab. In an alternative embodiment shown in Figures 27 and 28, a guide assembly 316 is constructed for use with a single second dimension electrophoresis gel cassette.

[0112] Referring to Figure 27, guide assembly 316 includes guide members 318 coupled together at the respective longitudinal ends 320 by support plates 322. Guide members 318 in this embodiment have a longitudinal dimension substantially equal to the longitudinal edge of the second dimension electrophoresis gel cassette.

[0113] Guide members 318 have an outer face 324 extending between a top end 326 and a bottom end 328 and defining a vertical dimension of guide member 318. Each guide member 318 has a body portion 330 with an inner face 332 defining guiding surfaces. A leg 334 extends downwardly from body 330. In the embodiment illustrated, support plates 322 are coupled to the longitudinal ends of legs 334. Legs 334 have a width less than a width of body 330 to form a ledge 336 extending inwardly. In one embodiment, each ledge 336 has a width corresponding to the thickness of the supporting plates of the gel cassette.

[0114] In a preferred embodiment, inner face 332 of each guide member 318 is inclined with respect to the vertical axis of guide members 318 and guide assembly 316. As shown in Figure 27, inner surfaces 332 converge in a downward direction to form a substantially V-shaped space 338 between guide members 318. In alternative embodiments, one of the inner surfaces of a guide member 318 is substantially vertical.

[0115] Assembly 316 is dimensioned to fit directly on the top edge 340 of a gel cassette 342 as shown in Figure 28. As in the previous

embodiments, cassette 342 includes an electrophoresis gel slab 344 supported between two support plates 346. Typically, ledge 336 of each guide member 318 has a width substantially equal to the thickness of support plates 346. In alternative embodiments, ledge 336 can have a width greater than the thickness of support plates 346 so that the bottom edge 348 of inner surface 332 overhangs the space 350 above gel 344.

[0116] As in the previous embodiments, the assembly 316 is positioned on cassette 342 and a cylindrical gel 352 from a first dimension electrophoresis gel tube unloaded between inner surfaces 332. Preferably, guide members are made of a non-stick material to enable gel 352 to slide easily into space 350.

[0117] While various embodiments of the invention have been illustrated, it will be understood by those skilled in the art that additions and modifications can be made without departing from the scope of the invention as set forth in the appended claims.